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Treatment response in enteric fever in an era of increasing antimicrobial resistance: an individual patient data analysis of 2,092 participants enrolled into four randomised controlled trials in Nepal

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Running title: Treatment of enteric fever in South Asia

Summary: This is the largest collection of enteric fever treatment data ever combined. The results, from trials conducted in Nepal since 2005, confirm that fluoroquinolones are failing for enteric fever treatment. The WHO enteric fever treatment guidelines should be modified.

Abstract

Background

Enteric fever, caused by Salmonella Typhi and Salmonella Paratyphi A, is the leading cause of bacterial febrile disease in South Asia.

Methods

Individual patient data from 2,092 subjects with enteric fever randomised into four trials in Kathmandu, Nepal was pooled. All trials compared gatifloxacin with a comparator drug: cefixime, chloramphenicol, ofloxacin, or ceftriaxone. Treatment outcomes were evaluated according to antimicrobial if S. Typhi/Paratyphi were isolated from blood. We additionally investigated the impact of changing bacterial antimicrobial susceptibility on outcome.

Results

Overall, 855 (41%) patients had either S. Typhi (n=581,28%) or S. Paratyphi A (n=274,13%) cultured from blood. There were 139 (6.6%) treatment failures with one death. Except for the last trial with ceftriaxone, the fluoroquinolone gatifloxacin was associated with equivalent or better fever clearance times and lower treatment failure rates in comparison to all other antimicrobials. However, we additionally found the minimum inhibitory concentrations (MIC) against fluoroquinolones have risen significantly since 2005 and were associated with increasing fever clearance times. Notably, all organisms were susceptible to ceftriaxone throughout the study period (2005-2014) and the MICs against azithromycin declined, confirming the utility of these alternative drugs for enteric fever treatment.

Conclusion

The World Health Organization and local government health ministries in South Asia still recommend fluoroquinolones as the drug of choice in the treatment of enteric fever. This policy should change based on the evidence provided here. Rapid diagnostics are urgently required given the large numbers of suspected enteric fever patients with a negative culture.

Key words: antimicrobial resistance, typhoid, enteric fever, Nepal, fluoroquinolone

Introduction

Enteric (typhoid) fever is a systemic infection caused by the Salmonella enterica serovars Typhi and Paratyphi A, B and C. Enteric fever is a significant cause of morbidity and mortality in low-income regions,[1] and was responsible for an estimated 12.2 million disability adjusted life years (DALYs) and >190,000 deaths globally in 2010.[2] The fatality rate of enteric fever is low (<1%), but is higher when antimicrobial therapy is delayed or unavailable.[3] Therefore, antimicrobials are essential for the clinical management of enteric fever. Chloramphenicol, ampicillin, and cotrimoxazole were first line enteric fever treatments until the early 1990s when the increasing incidence of multidrug resistant (MDR; defined as resistance to these three antimicrobial drugs) S. Typhi organisms led to the use of fluoroquinolones.[4,5] Yet organisms with reduced susceptibility against fluoroquinolones became a problem in Asia soon after their introduction.[6,7] Recent phylogeographic analyses documenting an on-going epidemic of a global AMR S. Typhi lineage suggest that the potential for regional or global dispersal of a lineage exhibiting resistance to fluoroquinolones is now a real threat.[8–10] In the absence of effective and accessible vaccines and lack of sanitation improvements, developing tailored antimicrobial therapy recommendations is critical to reduce morbidity and prevent disease transmission.

In Kathmandu, Nepal, S. Typhi and S. Paratyphi A are the most commonly isolated organisms from the blood of febrile adults and children.[11,12] Over the last decade we have conducted four randomised controlled trials (RCTs) evaluating enteric fever treatment in this endemic region.[13–16] The aim of this study was to use the largest collection of individual patient data assembled to date from enteric fever treatment trials to evaluate the effect of treatment drug on differences in clinical outcome between S. Typhi and S. Paratyphi A infections and those with blood culture negative enteric fever. We further sought to compare the antimicrobial susceptibility profiles over time between S. Typhi and S. Paratyphi A isolates and investigate their impact on outcome. Generating an in-depth understanding of trends and clinical implications of AMR enteric fever should guide policymakers and clinicians in decisions regarding treatment in an era of rapidly diminishing therapeutic options.

Methods

Ethical approval

Written informed consent was required for participation in all trials, which was provided by a parent or adult guardian if a patient was aged <18 years. The Ethics Committee of the Nepal Health Research Council (NHRC) and the Oxford Tropical Research Ethics Committee (OxTREC) of the United Kingdom provided ethical approval for all four studies.

Patient populations and study procedures

Individual patient data for this study were derived from four RCTs conducted at Patan Hospital in, Kathmandu, Nepal between 2005 and 2014, the methods and results of which have been described previously.[13–16] Patients presenting to the outpatient or emergency department with fever for >3 days with a clinical diagnosis of enteric fever (undifferentiated fever >38°C with no focus of infection) were eligible. Patients were excluded if they were pregnant or lactating, were under two years of age or weighed <10kg, showed any signs of complications (jaundice, shock, gastrointestinal bleeding), hypersensitivity to the relevant trial drugs or had been treated with a study drug in the week prior to attending hospital. The study procedures between the four trials were comparable, however there was several minor protocol differences between studies (outlined in Table S1).

Patients were randomly assigned to one of two arms in each trial. Each trial was composed of a gatifloxacin arm (10mg/kg/day, single dose orally for 7 days) and a comparator arm, which were: cefixime (20mg/kg/day, two doses orally for 7 days),[13] chloramphenicol (75mg/kg/day, four divided oral doses for 14 days),[14] ofloxacin (20mg/kg/day, two divided oral doses for 7 days)[15] and ceftriaxone (intravenous; 60mg/kg [2-13 years] or 2g/kg [≥14 years]).[16] Gatifloxacin was the constant comparator because it is inexpensive and given once daily.

Fever clearance time (FCT) was defined as the time from the first dose of a study drug until the

temperature dropped to ≤37.5°C and remained below this temperature for at least two days. The composite endpoint treatment failure summarised unfavourable outcomes and was defined as the occurrence of at least one of the following: persistent fever (FCT of more than seven days (trial 1 and 4) or more than ten days (trial 2 and 3) after treatment initiation), the need for rescue treatment, microbiological failure (blood culture positive for Salmonella) on day eight, relapse or disease-related complications within 31 days of treatment initiation or death. Blood was taken from all patients for microbiological culture on enrolment and on day eight for culture positive individuals or those with a potential relapse.

Microbiological investigations have been described previously.[13–16] Blood samples from adult patients were inoculated into media containing tryptone soya broth and sodium polyanethol sulphonate. For children, BacTEC Ped Plus/F bottles were used. Positive bottles were cultured onto MacConkey agar and presumptive Salmonella colonies were identified using biochemical tests and serotype-specific antisera. During all four trials, minimum inhibitory concentrations (MICs) were determined against the following antimicrobials unless otherwise noted: augmentin, ampicillin, amoxicillin, azithromycin (2006-2011), cefixime (2005), chloramphenicol, ciprofloxacin (2006-2014), ceftriaxone, gatifloxacin, naladixic acid, ofloxacin (2006-2014), cotrimoxazole (2006-2009, 2011-2014) and tetracycline by E-test (AB Biodisk, Sweden).

Statistical analyses

Data from the trials was combined and analysed using STATA v13.1 (College Station, Texas, USA). Plots were drawn in R v3.1.1 (R Foundation, Vienna, Austria) using the ggplot2 package. Demographics and clinical variables were tabulated and compared between serovars. Comparisons of clinical parameters between patient populations were structured as logistic regressions with the patient population (either culture positive/negative or S. Typhi/S. Paratyphi A) as the main covariate and adjustment for age stratum (binary: <16 years/≥16 years). Multivariable models with random effects were fitted to adjust for study

heterogeneity: (a) FCT was evaluated using Kaplan-Meier estimates and Cox proportional hazard models with treatment group, and age as covariates; (b) logistic regression was used to determine the odds of treatment failure between treatment arms, controlling for age and, (c) linear regression was used to evaluate the relationship between FCT and $\log_2$ MIC, also controlling for age. Generalized additive models (GAM, identity link, cubic spline) were used to examine potential non-linear trends of MIC over time...

Results

Baseline characteristics

Between 2005 and 2014 there were 2,118 patients with clinically suspected enteric fever randomised into four trials; data from 2,092 (99%) patients were evaluated (Figure 1). Of these, 855 (41%) were culture positive for either S. Typhi (n=581, 28%) or S. Paratyphi A (n=274, 13%). Throughout the study period there were 139 (6.6%) treatment failures including one death. The median patient age was 17 years (interquartile range [IQR]: 10-23); 66% were male (Table 1). There was no significant difference in age between the culture negative and culture positive patients, however S. Typhi patients were significantly younger (median: 16 years, IQR: 9-21) than S. Paratyphi A patients (median: 19.5 years, IQR: 13-24) ($p<0.001$) (Table 2). There was no difference in the sex distribution between culture positive/culture negative or S. Typhi/S. Paratyphi A populations (Table 2).

There were several significant differences in clinical history between patient populations after controlling for age (Table 2). Culture negative patients were significantly more likely to report coughing (40%) and vomiting (22%) than culture positive patients (31% and 17%, respectively). Culture positive patients, however, reported diarrhoea (24%) more often than culture negative patients (17%) in addition to a higher temperature (median: 39.0°C and 38.7°C, respectively). Amongst the culture positive patients, those with an S. Typhi infection were significantly more likely to report a history of anorexia (78%), coughing (33%) and diarrhoea (28%) in comparison to the S. Paratyphi A patients (71%, 25% and 15%, respectively) and

presented with higher temperatures (median: 39.0°C vs. 38.8°C). S. Paratyphi A patients were significantly more likely to report a history of previous typhoid illness (23%) compared to S. Typhi patients (12%). Additionally, there were several significant differences in haematology parameters between the culture negative/culture positive patients and the S. Typhi/S. Paratyphi A patients (Table 1), despite the majority of the values falling within normal ranges. AST and ALT were significantly elevated in the culture positive patients (median: 51 U/and median: 38 U/L, respectively) compared to culture negative patients (median: 42 U/L and median: 31 U/L, respectively).

Treatment failure

The number of patients failing treatment in each of the treatment arms is shown in Table 3. Rates of failure between antimicrobial treatment arms were largely similar when stratified by microbiological culture result with a few notable exceptions. In comparison to gatifloxacin, culture positive patients were significantly more likely to fail treatment when administered cefixime (OR: 10.7, 95%CI: 3.72-30.61, p<0.001). Culture negative patients were more likely to fail with cefixime (OR: 7.13, 95%CI: 2.82-18.0, p<0.001), ceftriaxone (OR: 19.3, 95%CI: 8.02-46.5, p<0.001) and chloramphenicol (OR: 3.67, 95%CI: 1.52-8.86, p=0.004) in comparison to gatifloxacin.

Fever clearance times

The FCTs of the various patient populations are shown in Figure 2 and Table 4. Amongst the culture positive patient population, S. Typhi patients treated with cefixime (HR: 0.36, 95%CI: 0.25-0.54, p<0.001) and ceftriaxone (HR: 1.53, 95%CI: 1.01-2.31, p=0.043) had significantly longer FCTs than S. Typhi patients treated with gatifloxacin. In the culture positive patients, those infected with S. Typhi also had significantly longer FCTs than S. Paratyphi A patients when treated with cefixime (HR: 2.18, 95%CI: 1.25-3.80, p=0.006) (Table 4). However, S. Paratyphi A infected patients had longer FCTs when treated with chloramphenicol compared to S. Typhi infected patients (HR: 0.069, 95%CI: 0.49-0.97, p=0.031). In

comparison to gatifloxacin, culture negative patients fared significantly worse when treated with cefixime (HR: 0.56, 95%CI: 0.43-0.71, p<0.001) and ceftriaxone (HR: 0.42, 95%CI: 0.31-0.57, p<0.001).

Antimicrobial susceptibility trends

As shown in Figure 3, the MICs for S. Paratyphi A were significantly higher than those for S. Typhi with all antimicrobials (p<0.001, Kruskal-Wallis), with the exception of cefixime (p=0.375). Figure 4 shows the MIC time trends by serovar, which were significantly non-linear over time for all antimicrobials in both serovars (GAM, p<0.001 with the exception of S. Paratyphi A/ciprofloxacin: p=0.052 and S. Paratyphi A/nalidixic acid: p=0.003). Most notably, the MICs against the fluoroquinolones rose significantly over time and the MICs against azithromycin declined between 2007 and 2010. Lastly, all isolates were susceptible to ceftriaxone throughout the study period.

The impact of antimicrobial resistance on clinical outcomes

Increasing MICs against fluoroquinolones led to longer FCT in S. Typhi patients. As shown in Figure 5, an increasing (log_2) MIC was associated with longer FCTs in patients treated with gatifloxacin (number of hours increase in FCT for each 2-fold increase in MIC (β)=8.1, 95%CI: 5.3-10.8, p<0.001) and ofloxacin (β=8.4, 95%CI: 2.2-14.5, p=0.008). Longer FCTs were also observed with increasing MICs against ciprofloxacin in S. Typhi patients treated with ofloxacin or gatifloxacin (β=6.88, 95%CI: 4.9-8.9, p<0.001). However, we found no significant association between FCT and (log_2) MIC against the fluoroquinolones in the S. Paratyphi A patients (all p>0.05). Additionally, there was no significant association between FCT and MIC for the other antimicrobials tested. Lastly, patients infected with a S. Typhi isolate that was non-susceptible to ciprofloxacin (MIC≥0.12μg/mL) were more likely to experience treatment failure (29/211, 13.7%) when treated with ofloxacin or gatifloxacin compared to patients infected with S. Typhi organisms susceptible to ciprofloxacin (MIC<0.12μg/mL) (2/79, 2.5%) (OR: 5.16, 95%CI: 1.1-23.2, p=0.033). Conversely, we did not identify a similar relationship in those infected with

S. Paratyphi A (8/149 [5.4%] vs. 1/6 [16.7%], OR: 0.32, 95%CI: 0.03-3.15, p=0.329), the majority of which exhibited reduced susceptibility against ciprofloxacin (MIC≥0.12μg/mL) (211/221, 96%).

Discussion

Enteric fever remains the leading cause of febrile bacterial illness in Kathmandu.[12] With alarming AMR rates, a lack of immunisation as a public health tool and slow sanitation improvements, tailored antimicrobial therapies for the prevailing AMR profiles are required. Using systematic longitudinal individual patient data we identified dynamic antimicrobial susceptibility profiles among S. Typhi and S. Paratyphi A isolates and a trend of increasing fluoroquinolone MICs correlating with poor outcome. This phenomenon was particularly apparent among S. Typhi patients. Although ceftriaxone was effective in treating culture confirmed enteric fever patients, we document poor clinical response in culture negative patients. These data suggest that careful consideration is required for antimicrobial therapy of patients with enteric fever. In addition, fluoroquinolones should not be recommended for empirical treatment of this infection in South Asia.[17]

By combining the largest number of enteric fever patients from a single location we were able to identify several notable differences in both clinical presentation and clinical response between S. Typhi and S. Paratyphi A patients. Previous work conducted at the same centre found the two serovars to be clinically indistinguishable,[18] we find that, after controlling for age, S. Typhi patients were more likely to report anorexia, diarrhoea and coughing and presented with a higher temperature.

The precise mechanism driving the variability in MICs over time for both S. Typhi and S. Paratyphi A against several antimicrobials throughout 2005-2014 is unknown, but may be determined by local prescribing practices. This hypothesis is consistent with notable declines in MDR organisms in both Nepal and India after fluoroquinolones became the first choice of treatment.[12,19,20] However, we predict a rapid rebound of MDR organisms with reversion to the prescribing of first line antimicrobials due to the

circulation of MDR plasmids in S. Typhi and other organisms.[8,21]

Our study period captured dynamic changes in MICs against fluoroquinolones, particularly amongst S. Typhi isolates in more recent years. Through whole genome sequencing we have determined that this rise in MIC is associated with the emergence of an H58 variant with mutations in the DNA gyrase gene (gyrA) and the DNA topoisomerase IV gene (parC).[10,16] Supporting these findings, we can conclusively show that FCTs and the rate of treatment failure increases with elevated MICs in S. Typhi patients treated with a fluoroquinolone, confirming results from small studies conducted elsewhere.[7,22] However, although S. Paratyphi A isolates had significantly higher MICs against all tested fluoroquinolones in comparison to S. Typhi, poor outcome was not significantly associated with increasing MIC. We suggest continued surveillance of S. Paratyphi A in the region to monitor for the emergence of high-level fluoroquinolone resistant organisms similar to trends in the S. Typhi population.

As highlighted in our most recent RCT, patients with suspected enteric fever who were blood culture negative were treated effectively with gatifloxacin, yet fared less well when treated with ceftriaxone.[16] The present analysis shows that ofloxacin also performs well in treating those with culture negative enteric fever, though due to the low sensitivity of blood culture for the detection of S. Typhi and S. Paratyphi A[23], it is likely ofloxacin may have been effective against undetected enteric fever cases. However, we have documented that a reasonable proportion (22%, 21/96) of patients enrolled in the third trial included in the present analysis[14] who were blood culture negative were serologically positive for murine typhus.[24] Doxycycline is considered the drug of choice for rickettsial infections, although it seems that fluoroquinolones may also have clinical activity.[24]

In 2003 the WHO published guidelines recommending azithromycin, ceftriaxone or cefixime for quinolone-resistant S. Typhi or S. Paratyphi A infections.[23] Azithromycin is safe and efficacious for the treatment of uncomplicated typhoid,[25,26] and although there are no current clinical MIC breakpoints, the

majority of isolates (88%) here were susceptible, using the previously suggested cut-off of <16µg/mL.[27] The low MICs against ceftriaxone and rapid FCTs throughout the study period indicate that this drug is likely to be effective for culture confirmed enteric fever in Nepal. The cost and parenteral route of administration, however, make ceftriaxone less suitable for patient treatment in low and middle income countries, particularly as 60-90% of enteric fever patients are treated as outpatients.[3] An alternative would be the oral third generation cephalosporin cefixime, however, our first trial, that compared gatifloxacin with cefixime had to be stopped early by the DSMB because of the high failure rate in the cefixime arm (26/77) compared to gatifloxacin arm (5/92; OR ~9), despite all strains being cefixime susceptible.[13] Our analysis supports a recommendation for azithromycin or ceftriaxone for culture confirmed enteric fever and in the absence of rapid diagnostics for rickettsial infections,[28] a combination of ceftriaxone and doxycycline in culture negative febrile patients in this setting.[16] However, identification of Extended Spectrum Beta Lactamase (ESBL) producing S. Paratyphi A in India again suggests vigilance is required.

Our study has limitations. First, the poor diagnostic sensitivity of blood culture may lead to a misclassification of a significant number of patients, though a proportion of culture negatives are likely to be positive for Rickettsia spp.; this was not directly assessed.[24] Furthermore, by combining patients from individual RCTs with some differing definitions, the data became non-randomised, however we included a random effect of study to account for heterogeneity between studies and controlled for age. Therefore, strong associations, such as odds of treatment failure between cefixime and gatifloxacin in culture positive patients, may be reduced with the larger, non-randomised data. Additionally, we were unable to access pharmacy records to evaluate the relationship of prescribing patterns for febrile patients and MICs against common antimicrobials. Notwithstanding these limitations, these results from this largest collection of trials with patient recruitment spanning a decade in an endemic location with a high burden of disease will help to inform therapy recommendations.

In conclusion, poor sanitation, low vaccine uptake and the emergence of extensive ciprofloxacin-resistant S. Typhi in Kathmandu suggest that appropriate antimicrobial usage policies are required for limiting morbidity, mortality and transmission. In this large evaluation, we document shifting antimicrobial susceptibility profiles; an association between poor treatment outcome and S. Typhi MICs in patients treated with a fluoroquinolone and again highlight the need for better diagnostics for febrile diseases in this setting. We reiterate that fluoroquinolones should not be recommended for the empirical treatment of enteric fever in South Asia,[8,29] and advocate the use of azithromycin or ceftriaxone, alongside surveillance for changes in AMR profiles.

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Declaration of interests

The authors declare no competing interests.

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Figure 1. Enrolment of patients into enteric fever treatment trials in Nepal

Flow chart showing enrolment of patients into the four individual randomized controlled trials according to antimicrobial treatment and blood culture result

Figure 2. Fever clearance time by treatment arm and culture result

Fever clearance time (in days) is shown for S. Typhi, S. Paratyphi A and culture negative patients. Colours indicate the different treatment arms. CFX: cefixime; CHL: chloramphenicol; CRO: ceftriaxone; GAT: gatifloxacin; OFX: ofloxacin.

Figure 3. Distribution of MICs against antimicrobials for S. Typhi and S. Paratyphi A

MICs shown on a $\log_2$ scale against 12 antimicrobials for S. Typhi (blue) and S. Paratyphi A (orange). Lower, middle and upper horizontal dashed lines represent the current CLSI cut-offs for susceptible/intermediate and intermediate/resistant, respectively [30].

Figure 4. MICs over time for S. Typhi and S. Paratyphi A

MICs shown on a $\log_2$ scale for eight antimicrobials over 2005-2014. S. Typhi are shown in blue and S. Paratyphi A are shown in orange. The smoothed line is derived from the generalized additive model showing a non-linear increase in MICs over time, with the shaded region showing the 95% confidence interval. Lower, middle and upper horizontal dashed lines represent the current CLSI cut-offs for susceptible/intermediate and intermediate/resistant, respectively [30].

Figure 5. Fever clearance time and MIC against fluoroquinolones for S. Typhi and S. Paratyphi A

Fever clearance time in days is shown plotted against $\log_2$ MIC for gatifloxacin (left) and ofloxacin (right). S. Typhi isolates are shown in blue and S. Paratyphi A isolates are shown in orange. The lines represent the best-fit linear model with 95% confidence interval shown by the shaded region.

Table 1. Baseline characteristics of patients enrolled in four enteric fever treatment trials

Characteristic	Trial 1		Trial 2		Trial 3		Trial 4		Total	
	N	n (%) or median (IQR)	N	n (%) or median (IQR)	N	n (%) or median (IQR)	N	n (%) or median (IQR)	N	n (%) or median (IQR)
Age (yr)	382	17 (9-23)	844	16 (9-22)	623	17 (9-23)	239	19 (15-23)	2,088	17 (10-23)
Male sex	382	247 (64.7)	844	540 (64.0)	627	406 (64.8)	239	180 (75.3)	2,092	1,373 (65.6)
Weight (kg)	382	45 (24-53)	842	42 (21-52)	627	45 (25-54)	237	50 (40-56)	2,088	45 (24-53)
Duration of illness before admission (days)	382	5 (3-6)	844	5 (4-7)	625	5 (4-7)	180	5 (4-7)	2,031	5 (4-7)
Treatment with antimicrobials in the past 2 weeks	379	238 (62.8)	724	694 (95.9)	623	428 (68.7)	210	109 (51.9)	1,936	1,469 (75.9)
Previous history of typhoid	382	61 (16.0)	844	138 (16.4)	626	103 (16.5)	238	37 (15.5)	2,090	339 (16.2)
Family history of typhoid	382	62 (16.2)	844	140 (16.6)	625	164 (26.2)	239	35 (14.6)	2,090	401 (19.2)
Typhoid vaccination	382	2 (0.5)	844	0 (0)	625	0 (0)	238	11 (4.6)	2,089	13 (0.6)
Temperature at admission (°C)	379	38.9 (38.3-39.5)	844	38.9 (38.2-39.4)	626	38.6 (38.2-39.0)	235	38.8 (38.3-39.4)	2,084	38.8 (38.2-39.4)
Headache	382	370 (96.9)	844	749 (88.7)	627	541 (86.3)	239	211 (88.3)	2,092	1,871 (89.4)
Anorexia	382	289 (75.7)	844	632 (74.9)	627	455 (72.6)	239	173 (72.4)	2,092	1,549 (74.0)
Abdominal pain	382	32 (8.4)	844	33 (3.9)	626	25 (4.0)	235	62 (26.4)	2,087	152 (7.3)
Cough	382	142 (37.2)	844	277 (32.8)	627	246 (39.2)	239	91 (38.1)	2,092	756 (36.1)
Nausea	382	132 (34.6)	844	258 (30.6)	627	174 (27.8)	239	124 (51.9)	2,092	688 (32.9)
Vomiting	382	57 (14.9)	844	172 (20.4)	627	118 (18.8)	239	69 (28.9)	2,092	416 (19.9)
Diarrhoea	382	86 (22.5)	844	161 (19.1)	627	105 (16.7)	239	59 (24.7)	2,092	411 (19.6)
Constipation	382	41 (10.7)	844	105 (12.4)	627	79 (12.6)	239	31 (13.0)	2,092	256 (12.2)
Hepatomegaly	382	19 (5.0)	844	113 (13.4)	626	7 (1.1)	231	0 (0)	2,083	139 (6.7)
Splenomegaly	382	35 (9.2)	844	119 (14.1)	626	6 (1.0)	231	2 (0.9)	2,083	162 (7.8)
Haematocrit (%)	370	40 (37-44)	831	39 (36-43)	624	38 (36-42)	235	39 (36-43)	2,060	39 (36-43)
Leucocyte count ($x10^9$/L)	370	7.0 (5.5-9.0)	831	6.3 (5.0-8.1)	624	6.0 (4.8-7.7)	239	5.9 (4.7-7.3)	2,064	6.3 (5.0-8.0)
Platelet count ($x10^9$/L)	356	190 (160-235)	800	190 (164-226)	615	174 (145-216)	239	168 (150-209)	2,010	184 (153-220)
AST (U/L)	373	47 (36-62)	835	45 (34-61)	624	47 (34-67)	233	49 (36-70)	2,065	46 (35-65)
ALT (U/L)	373	33 (24-48)	836	29 (20-43)	624	37 (28-53)	234	45 (31-63)	2,067	34 (24-50)
S. Typhi isolated	382	119 (31.2)	844	249 (29.5)	627	132 (21.1)	239	81 (33.9)	2,092	581 (27.8)
S. Paratyphi A isolated	382	50 (13.1)	844	103 (12.2)	627	86 (13.7)	239	35 (14.6)	2,092	274 (13.1)
No growth or culture negative	382	213 (55.8)	844	492 (58.3)	627	409 (65.2)	239	123 (51.5)	2,092	1,237 (59.1)

Trials: 1 – gatifloxacin/cefixime [13], 2 – gatifloxacin/chloramphenicol [14], 3 – gatifloxacin/ofloxacin [15], 4 – gatifloxacin/ceftriaxone [16]

Table 2. Demographic and clinical characteristics of culture negative, culture positive, Salmonella Typhi and Salmonella Paratyphi A patients

Characteristic	Culture negative		Culture positive		p value ^	S. Typhi		S. Paratyphi A		p value ^
	N	n (%) or median (IQR)	N	n (%) or median (IQR)		N	n (%) or median (IQR)	N	n (%) or median (IQR)	
Age (yr)*	1,236	17 (9-24)	852	17 (10-22)	0.692	578	16 (9-21)	274	19.5 (13-24)	<0.001
Male sex*	1,237	818 (66.1)	855	555 (64.9)	0.565	581	373 (64.2)	274	182 (66.4)	0.525
Weight (kg)	1,234	44 (23-54)	854	46 (25-53)	0.854	580	43.5 (22-52)	274	49 (38-55)	<0.001
Duration of illness before admission (days)	1,203	5 (4-7)	828	5 (4-7)	0.500	565	5 (4-7)	263	5 (4-6)	0.102
Treatment with antimicrobials in the past 2 weeks	1,146	861 (75.1)	790	608 (77.0)	0.330	532	414 (77.8)	258	194 (75.2)	0.440
Previous history of typhoid	1,236	208 (16.8)	854	131 (15.3)	0.276	581	68 (11.7)	273	63 (23.1)	<0.001
Family history of typhoid	1,236	242 (19.6)	854	159 (18.6)	0.657	580	107 (18.4)	274	52 (19.0)	0.400
Typhoid vaccination	1,234	9 (0.7)	855	4 (0.5)	0.511	581	1 (0.2)	274	3 (1.1)	0.073
Temperature at admission (°C)	1,233	38.7 (38.1-39.2)	851	39 (38.4-39.5)	<0.001	577	39 (38.5-39.5)	274	38.8 (38.2-39.2)	<0.001
Headache	1,237	1098 (88.8)	855	773 (90.4)	0.348	581	518 (89.2)	274	255 (93.1)	0.237
Anorexia	1,237	903 (73.0)	855	646 (75.6)	0.190	581	451 (77.6)	274	195 (71.2)	0.036
Abdominal pain	1,237	479 (38.7)	855	258 (30.2)	0.067	581	261 (44.9)	274	97 (35.4)	0.061
Cough	1,237	495 (40.0)	855	261 (30.5)	<0.001	581	193 (33.2)	274	68 (24.8)	0.011
Nausea	1,237	394 (31.9)	855	294 (34.4)	0.310	581	198 (34.1)	274	96 (35.0)	0.853
Vomiting	1,237	271 (21.9)	855	145 (17.0)	0.010	581	106 (18.2)	274	39 (14.2)	0.324
Diarrhoea	1,237	210 (17.0)	855	201 (23.5)	<0.001	581	161 (27.7)	274	40 (14.6)	<0.001
Constipation	1,237	154 (12.4)	855	102 (11.9)	0.775	581	63 (10.8)	274	39 (14.2)	0.114
Hepatomegaly	1,234	84 (6.8)	849	55 (6.5)	0.847	578	40 (6.9)	271	15 (5.5)	0.804
Splenomegaly	1,234	85 (6.9)	849	77 (9.1)	0.069	578	48 (8.3)	271	29 (10.7)	0.224
Haematocrit (%)	1,219	39 (36-43)	841	39 (36-43)	0.573	569	39 (35-43)	272	40 (37-44)	0.006
Leucocyte count (x109/L)	1,220	6.4 (5.0-8.6)	844	6.1 (4.9-7.5)	<0.001	572	6.2 (4.9-7.5)	272	5.8 (4.8-7.2)	0.528
Platelet count (x109/L)	1,187	187 (157-229)	823	180 (150-210)	0.002	555	180 (151-214)	268	180 (150-210)	0.469
AST (U/L)	1,220	42 (32-59)	845	51 (40-69)	<0.001	573	54 (42-71)	272	47 (37.5-66)	0.023
ALT (U/L)	1,220	31 (21-46.5)	847	38 (28-53)	<0.001	575	39 (28-53)	272	36 (28-49.5)	0.564

^p-values derived from logistic regression (categorical variables) or linear regression (continuous variables) with outcome characteristic of interest and a covariate of culture positivity or serovar, controlling for age (<15years/≥16 years); *p-values derived using Fisher's exact test for categorical data and the Kruskal-Wallis test for continuous data (not controlled for age)

Table 3. Proportion of enteric fever patients with treatment failure by culture result and treatment

Treatment arm	Culture negative		Culture positive		S. Typhi		S. Paratyphi A	
	Total	n (%)	Total	n (%)	Total	n (%)	Total	n (%)
Gatifloxacin	617	9 (1.5)	440	36 (8.2)	298	26 (8.7)	142	10 (7.0)
Cefixime	105	10 (9.5)	77	26 (33.8)	54	19 (35.2)	23	7 (30.4)
Ceftriaxone	65	15 (23.1)	54	4 (7.4)	38	3 (7.9)	16	1 (6.3)
Chloramphenicol	243	12 (4.9)	175	14 (8.0)	125	11 (8.8)	50	3 (6.0)
Ofloxacin	207	5 (2.4)	109	8 (7.3)	66	7 (10.6)	43	1 (2.3)

Table 4. Fever clearance time (FCT) (in hours) for four enteric fever patient populations by treatment

Population	Culture negative			Culture positive			S. Typhi			S. Paratyphi A		
	N	Median FCT (IQR)	range	N	Median FCT (IQR)	range	N	Median FCT (IQR)	range	N	Median FCT (IQR)	range
Overall	1178	41.3 (18.2-71.3)	1.0-425.5	810	92.7 (65.3-124.7)	1.0-496.0	549	92.0 (66.4-125)	1.0-496.0	261	94.4 (56.1-122.8)	1.0-349.0
Treatment arm												
GAT	585	39.1 (17.0-68.0)	1.0-285.9	416	90.9 (64.3-116.9)	1.0-349.0	283	90.8 (67.4-117.3)	1.0-309.6	133	91.9 (55.8-116.0)	6.8-349.0
CFX	96	66.5 (18.5-134.5)	4.0-324.0	69	134.0 (82.0-205.0)	16.0-496.0	47	140.0 (96.0-232.0)	40.0-496.0	22	100.0 (81.0-164.0)	16.0-214.0
CRO	62	102.3 (31.5-161.5)	1.0-354.3	54	73.5 (46.0-112.8)	7.8-232.8	38	82.6 (54.0-117.5)	7.8-215.4	16	53.1 (43.3-83.0)	7.8-232.8
CHL	239	41.5 (20.2-68.7)	1.0-304.5	169	94.2 (65.2-136.3)	2.8-327.4	120	89.8 (65.2-121.7)	2.8-327.4	49	114.7 (63.4-151.6)	4.4-262.8
OFX	196	36.8 (17.9-66.4)	1.0-425.5	102	94.8 (56.0-122.3)	1.0-311.8	61	89.8 (48.0-115.4)	3.6-189.8	41	104.4 (71.5-141.6)	1.0-311.8

GAT: gatifloxacin; CFX: cefixime; CRO: ceftriaxone; CHL: chloramphenicol; OFX: ofloxacin; IQR: interquartile range

Figure 1

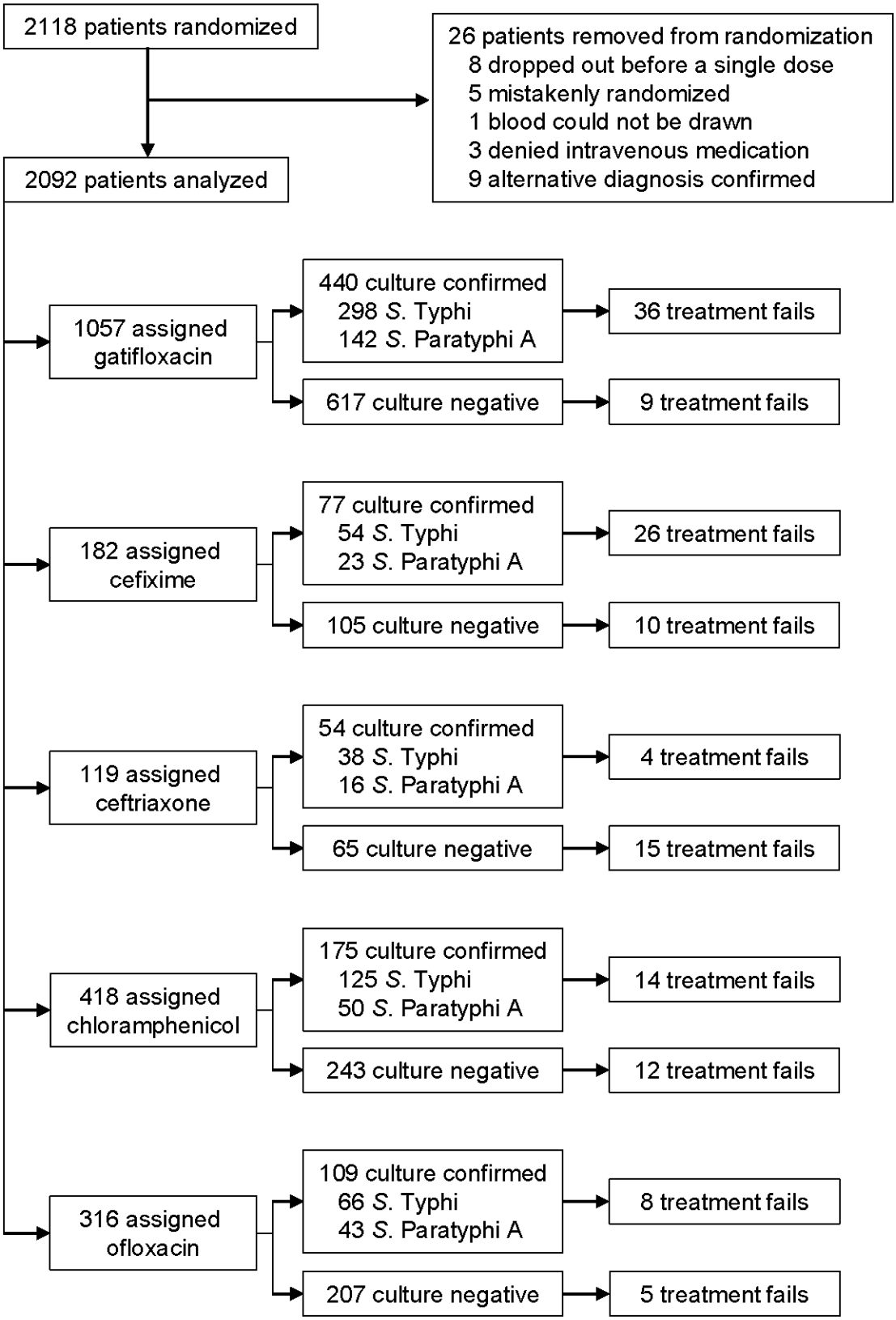

2118 patients randomized
26 patients removed from randomization
8 dropped out before a single dose
5 mistakenly randomized
1 blood could not be drawn
3 denied intravenous medication
9 alternative diagnosis confirmed
2092 patients analyzed
1057 assigned gatifloxacin
440 culture confirmed
298 S. Typhi
142 S. Paratyphi A
36 treatment fails
617 culture negative
9 treatment fails
182 assigned cefixime
77 culture confirmed
54 S. Typhi
23 S. Paratyphi A
26 treatment fails
105 culture negative
10 treatment fails
119 assigned ceftriaxone
54 culture confirmed
38 S. Typhi
16 S. Paratyphi A
4 treatment fails
65 culture negative
15 treatment fails
418 assigned chloramphenicol
175 culture confirmed
125 S. Typhi
50 S. Paratyphi A
14 treatment fails
243 culture negative
12 treatment fails
316 assigned ofloxacin
109 culture confirmed
66 S. Typhi
43 S. Paratyphi A
8 treatment fails
207 culture negative
5 treatment fails

Figure 2

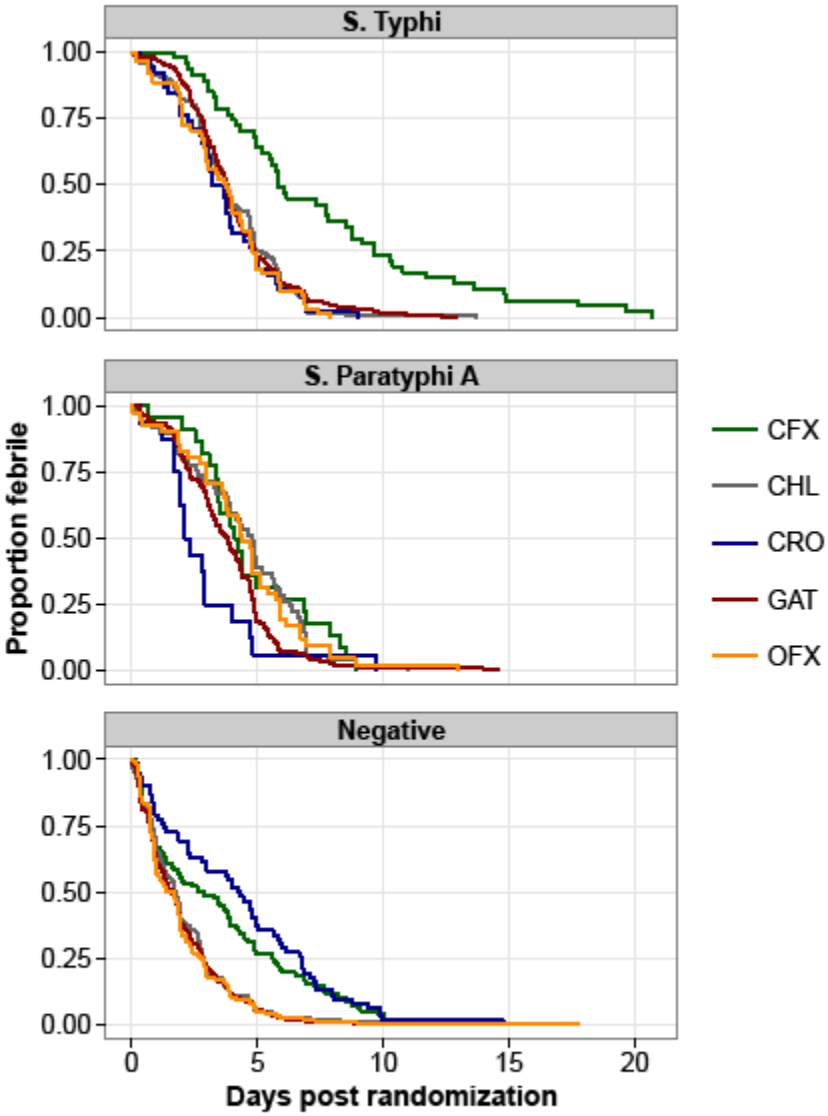

S. Typhi
S. Paratyphi A
Negative
Proportion febrile
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
Days post randomization
CFX
CHL
CRO
GAT
OFX

Figure 3

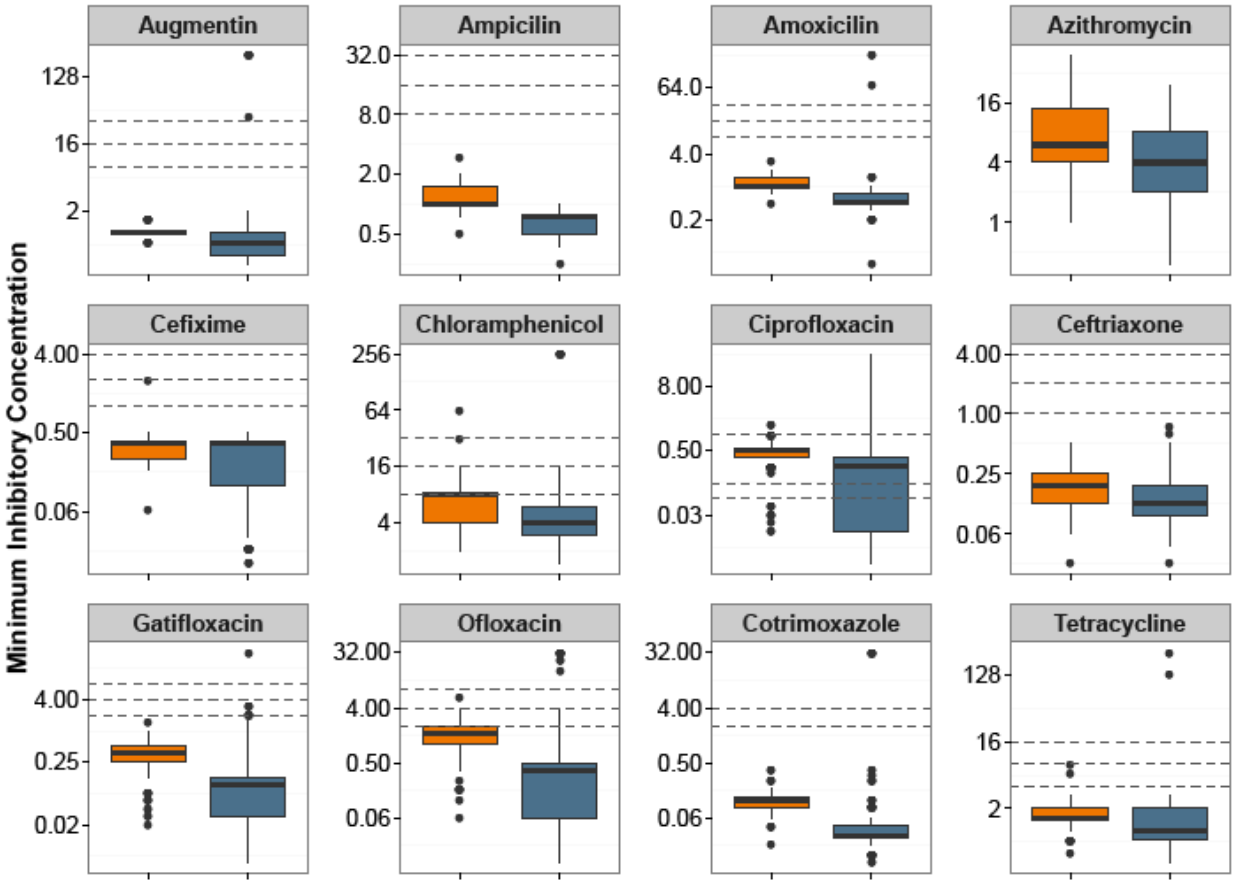

Minimum Inhibitory Concentration
Augmentin
128
16
2
Ampicilin
32.0
8.0
2.0
0.5
Amoxicilin
64.0
4.0
0.2
Azithromycin
16
4
1
Cefixime
4.00
0.50
0.06
Chloramphenicol
256
64
16
4
Ciprofloxacin
8.00
0.50
0.03
Ceftriaxone
4.00
1.00
0.25
0.06
Gatifloxacin
4.00
0.25
0.02
Ofloxacin
32.00
4.00
0.50
0.06
Cotrimoxazole
32.00
4.00
0.50
0.06
Tetracycline
128
16
2

Figure 4

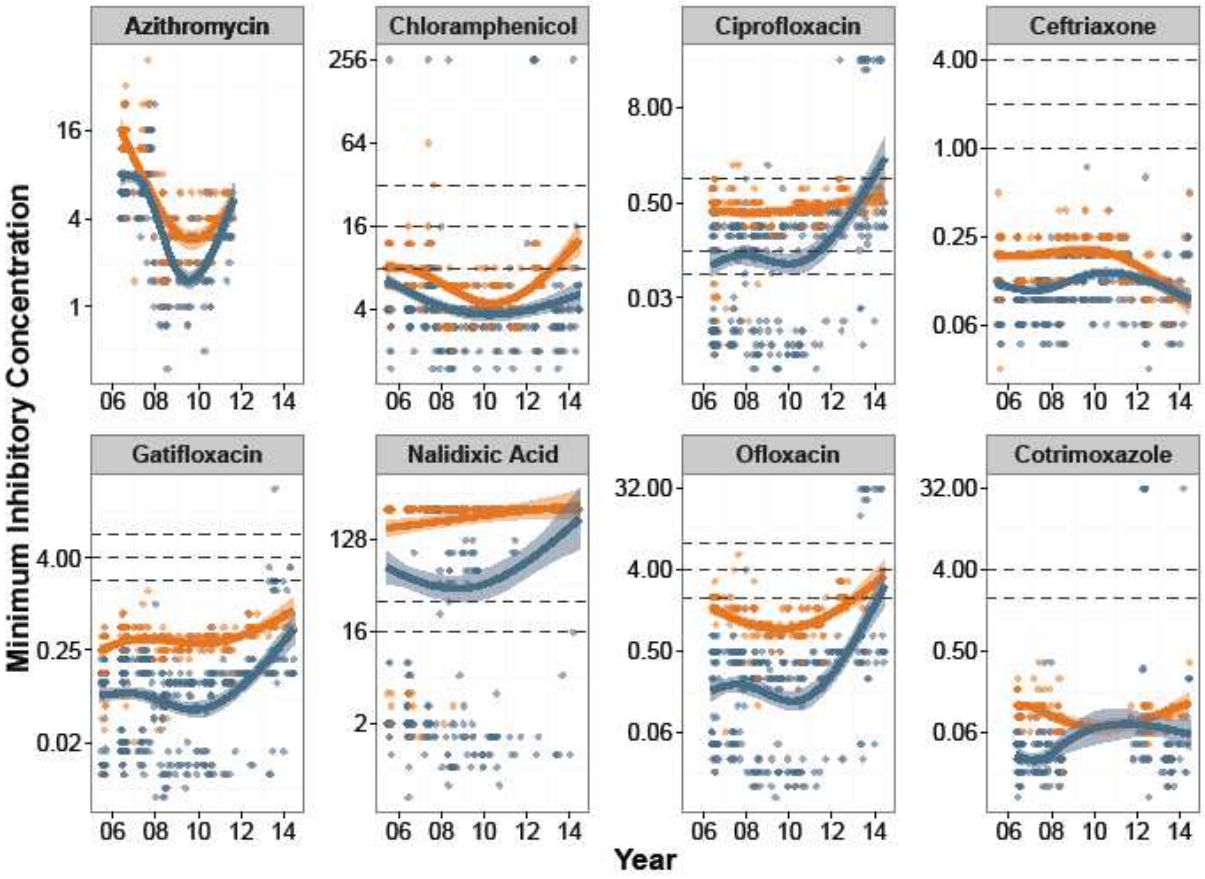
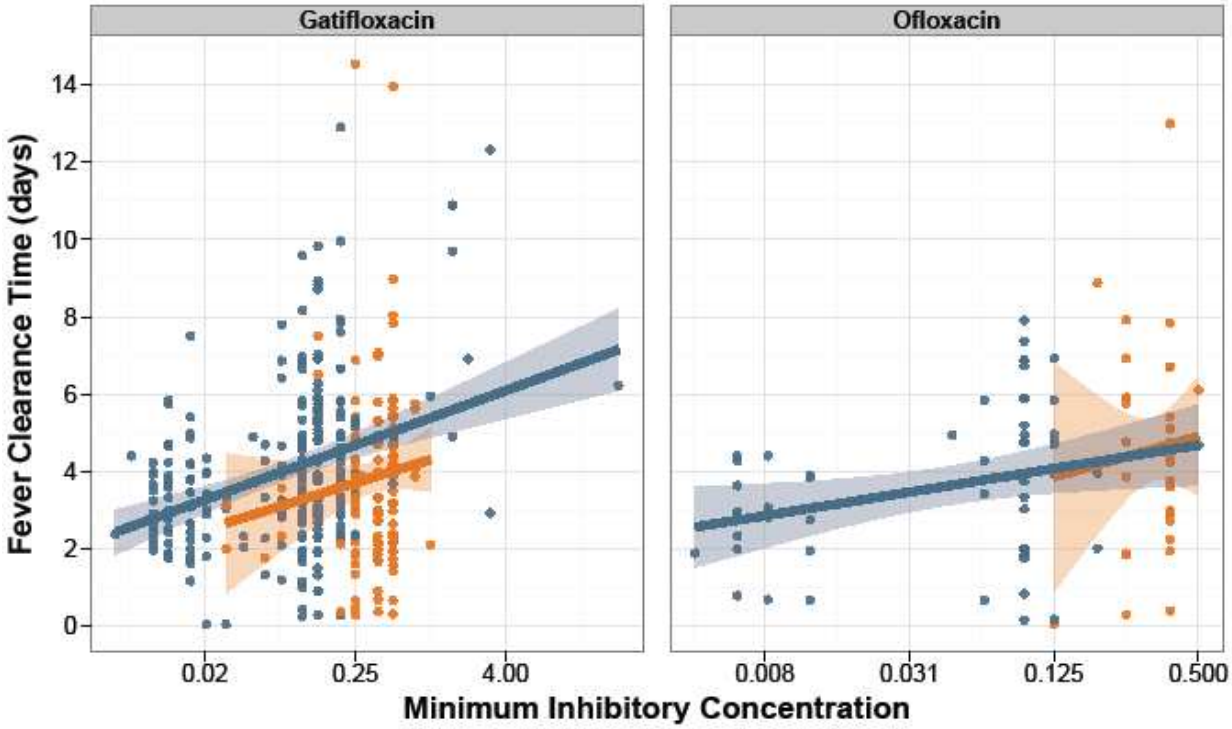

Azithromycin
Chloramphenicol
Ciprofloxacin
Ceftriaxone
Gatifloxacin
Nalidixic Acid
Ofloxacin
Cotrimoxazole
Minimum Inhibitory Concentration
Year
06 08 10 12 14

Figure 5


Gatifloxacin
Ofloxacin
Fever Clearance Time (days)
0
2
4
6
8
10
12
14
0.02
0.25
4.00
0.008
0.031
0.125
0.500
Minimum Inhibitory Concentration